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(54) Gamma interferon composition.

(57) Gamma interferon obtainable from the human leukocytes, which is unstable even during lyophilization and storage in solid state, can be stabilized by addition of albumin and/or a sugar such as glucose, mannose, galactose, fructose, sucrose, mannitol and xylitol. A stable, lyophilized gamma interferon composition is prepared by lyophilizing its aqueous solution containing the abovementioned stabilizer without lowering its activity.

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GAMMA INTERFERON COMPOSITION

This invention relates to a stable gamma interferon (hereinafter referred to as IFN-γ) composition.

IFN-γ is a glycoprotein having a molecular weight of about 20,000 to 40,000 which is produced when human leukocytes are stimulated with a mitogen such as phytohemagglutinin (hereinafter referred to as PHA) or concanavarin A (hereinafter referred to as Con-A) used as an inducer.

IFN-γ has a marked immunosuppressive, antiviral and anticellular action; further, it gives a synergistic effect to the activity of IFN-α and IFN-β; moreover, it has 10 to 100 times as high an antiproliferation effect against tumor cells as that of IFN-α or IFN-β. Accordingly, a great expectation is placed on the clinical effect of IFN-γ as a medicine.

But IFN-γ is unstable to acid conditions (for example, pH of about 2) or to heat. It also has a poor storage stability in solution. Hence, when IFN-γ is to be used as a medicine, it would be deemed desirable to make a lyophilized product of it; but actually, even during the lyophilization step it loses its activity. Accordingly, it is necessary to stabilize IFN-γ beforehand by some means.

Under these circumstances, the inventors, after extensive studies, have found out that, when a definite amount of a sugar or an albumin is made to exist together with IFN-γ, the IFN-γ is stabilized; no inactivation takes place during the lyophilization of the aqueous solution containing IFN-γ; moreover the storage stability of the dry preparation formed by lyophilization is improved. This invention has been accomplished on the basis of above finding.

Thus, this invention relates to a stable IFN-γ composition which comprises IFN-γ and a sugar or an albumin in an amount sufficient for stabilizing IFN-γ.

The IFN-γ to be used in this invention is usually that which is obtained by purification by chromatography using an antigen column or the like of the crude IFN-γ produced by stimulation of human leukocytes with a mitogen such as PHA and Con-A used as an inducer. But a wide variety of other IFN-γ including those produced by E.coli, yeast and the like by virtue of genetic engineering may also be used irrespective of their origin.

The sugars to be used in this invention include not only a normal sugar such as monosaccharides and disaccharides, but also a sugar alcohol and the mixtures thereof. There may be mentioned, as examples of monosaccharides, glucose, mannose, galactose and fructose; as those of disaccharides, sucrose, maltose

and lactose; and as those of sugar alcohols, mannitol and xylitol. A sugar may be used in admixture with albumin.

The albumin to be used in this invention is preferably of human origin because of the antigenicity problem. Such albumins can be used without further limitation so long as they have been purified for medical use. The purity is preferably such that they contain at least 80% of albumin as determined by electrophoresis analysis. As the methods for obtaining human-origin albumin, there may be mentioned, for example, the ethanol fractionation method (Japanese Patent Publication Nos. 2869/72 and 5297/60) and the method of heating a fraction thereof in the presence of an organic acid (Japanese Patent Publication Nos. 1604/68 and 401321/76). Particularly, albumins which have been subjected to heat treatment (preferably at 60°C for about 10 hours) to effect inactivation of hepatitis virus and the like are preferably used.

The amount of sugar or albumin sufficient for stabilizing IFN-γ is in a ratio of at least 5 mg of sugar or at least 3 mg of albumin per $1 \times 10^2 - 1 \times 10^7$ units of IFN-γ, and preferably 10 mg of sugar or 5-10 mg of albumin per $1 \times 10^4 - 1 \times 10^5$ units of IFN-γ. This compounding ratio is applicable to the stabilization of IFN-γ in the form of either solid or aqueous solution. In the lyophilization of an aqueous solution of IFN-γ, addition of sugar to a concentration of at least 5 mg/ml,

preferably 10 mg/ml of sugar or at least 3 mg/ml, preferably 5 mg/ml of albumin in the solution is sufficient to achieve the stabilization of IFN-γ, irrespective of the content of IFN-γ in the solution.

The lyophilization in the presence of sugar or albumin may be performed, for example, as follows. An aqueous solution containing purified IFN-γ is adjusted to pH 5 to 9; sugar or albumin is added to the solution in a stabilizing amount mentioned above; the resulting aqueous solution is filtered aseptically, dispensed in portions into vials, and then lyophilized in a conventional manner.

The lyophilized preparation of IFN-γ thus obtained has preferably a composition approximately corresponding to 10,000 - 500,000 units (preferably 50,000 - 500,000 units) of IFN-γ, 5-20 mg (preferably

10-20 mg) of sugar or 3-20 mg (preferably 5-20 mg) of alubmin and about 9 - about 18 mg of sodium chloride.

The IFN-γ preparation provided by the method of this invention is administered orally or parenterally. The dose varies depending upon the diseases to be treated, administration routes and so forth. When used as injections, for example, it is usually made up into a solution containing about 1-10% (w/v) of IFN-γ with distilled water or physiological saline solution for injection, and administered intravenously or intramuscularly at a dose of 2×10^3 - 1×10^4 units of IFN-γ /kg body weight depending on the symptoms of patients. In such case albumin would be administered in an amount of 0.1-0.4 mg/kg and sugar in an amount of 0.2-0.4 mg/kg.

This invention will be described in more detail below with reference to Examples and Experimental Examples, but it is in no way limited thereto.

Examples 1 to 9

IFN-γ produced in leukocytes with PHA used as the stimulant was purified by subjecting it to an affinity chromatography using Sepharose-monoclonal IFN-γ antibody as column and 2M KSCN solution (pH 8) as eluent to give a specific activity of 10^6 U/mg or more. The purified IFN-γ was dialyzed against a phosphate-sodium chloride buffer solution, pH 7. Then, a series of solution groups of various titers was prepared which con-

tained the dialyzed IFN-γ in concentrations of 1 x 10^2 units/ml, 1 x 10^3 units/ml, 1 x 10^4 units/ml, 1 x 10^5 units/ml and 1 x 10^6 units/ml. To each of the solution groups, was added glucose (Example 1), mannose (Example 2), galactose (Example 3), fructose (Example 4), sucrose (Example 5), maltose (Example 6), lactose (Example 7), mannitol (Example 8) or xylitol (Example 9) to a concentration of 10 mg/ml, respectively. The solutions were then filtered aseptically, dispensed in 2 ml portions into vials of 10 ml volume, and lyophilized at temperatures finally reaching 25°C.

The water content of the resulting lyophilized product determined according to "the general method, criterion for biological preparations" was found to be about 0.2% for every specimen. All of the lyophilized products dissolved immediately on addition of 2 ml of distilled water for injection and gave a clear, colorless solution. The amounts of remaining IFN-γ determined with these solutions all showed no difference from those before lyophilization.

Example 10.

Example 1 was repeated, provided that in place of glucose to a concentration of 10 mg/ml albumin was added to a concentration of 5 mg/ml.

The resulting lyophilized product has a water content of about 2% and dissolved immediately.

The property of the solution and the activity of remaining IFN-γ therein are same as in Example 1.

Experimental Exampl 1

A series of experiments was carried out to confirm the stabilizing effect according to this invention. To a solution of purified IFN-γ (1 x 10^4 units/ml; the titer being determined according to the CPE inhibition method in FL-Sindvis Virus system), was added glucose, sucrose, or mannitol to concentrations of 1 mg/ml, 5 mg/ml and 10 mg/ml, respectively, and the resulting solutions were lyophilized. The titer of the product was determined immediately before lyophilization, immediately after lyophilization, and after storage of 6 months at room temperature. The percentage of remaining activity relative to the titer immediately after the addition of sugar was as shown in Table 1.

Table 1

Sugar		Remaining ratio of IFN-γ activity (%)		
Kind	Added amount	Immediately before lyophilization	Immediately after lyophilization	6 Months' storage at room temperature
Glucose	1	98	40	19
	5	102	82	76
	10	101	93	89
Sucrose	1	99	46	15
	5	97	86	78
	10	100	96	90
Mannitol	1	100	37	14
	5	101	83	75
	10	99	91	87
None		100	-	32

Experimental Example 2

A series of experiments was carried out to confirm the stabilizing effect according to this invention. To a solution of purified IFN-γ (1×10^4 units/ml; the titer being determined according to the CPE inhibition method in FL-Sindvis Virus system), was added human

serum albumin to various concentrations (0 to 20 mg/ml as shown in Fig. 1), and the resulting solutions were lyophilized. The titer of the lyophilized product was determined immediately after lyophilization (indicated by symbol ○) and after storage of 6 months at room temperature (indicated by symbol ●). The percentage of remaining activity relative to the titer immediately after the addition of human serum albumin was as shown in Fig. 1.

What is claimed is:

1. A stable gamma interferon composition comprising a gamma interferon and an amount sufficient for stabilizing the gamma interferon of a sugar or an alubumin.

2. The stable gamma interferon composition of Claim 1, wherein the sugar is a monosaccharide, a disaccharide or a sugar alcohol.

3. The stable gamma interferon composition of Claim 2, wherein the sugar is glucose, mannose, galactose, fructose, sucrose, maltose, lactose, mannitol or xylitol.

4. The stable gamma interferon composition of any of Claims 1-3, wherein the amount of the sugar is at least 5 mg per $1 \times 10^2 - 1 \times 10^7$ units of the gamma interferon.

5. The stable gamma interferon composition of Claim 4, wherein the amount of the sugar is 10 mg per $1 \times 10^4 - 1 \times 10^5$ units of the gamma interferon.

6. The stable gamma interferon composition of any preceding Claim, which is in the form of a lyophilized solid.

7. The stable gamma interferon composition of any of Claims 1-5, which is in the form of an aqueous solution.

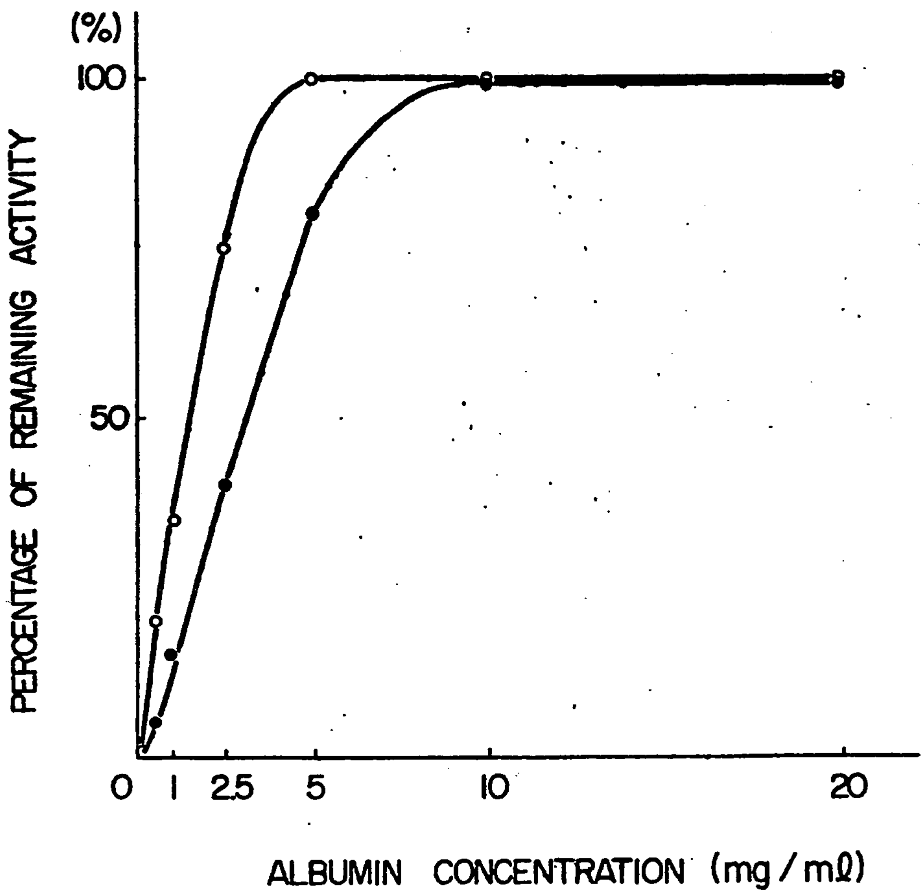
8. The stable gamma interferon composition of any of Claims 1, 6 and 7, wherein the amount of albumin is at least 3 mg per $1 \times 10^2 - 1 \times 10^7$ units of the gamma interferon.

9. The stable gamma interferon composition of

Claim 8, wherein the amount of albumin is 5-10 mg per $1 \times 10^4 - 1 \times 10^5$ units of the gamma interferon.

10. A process for producing the stable gamma interferon composition of Claim 6, which comprises lyophilizing the stable gamma interferon composition of Claim 7.

FIG. 1


(%)
100
50
PERCENTAGE OF REMAINING ACTIVITY
0
1
2.5
5
10
20
ALBUMIN CONCENTRATION (mg / mℓ)

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